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ANNUAL RESEARCH PROGRESS REPORT

(FY 2009)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58203

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** JAY J CAO

Start Date: 08/01/2005 **Term Date:** 07/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Enhance the quality of life through establishing mineral intakes that support optimal bone health. Specifically, determine the amount of dietary calcium needed to maximize calcium retention and minimize bone resorption in postmenopausal women; determine the extent to which dietary protein, specific mineral elements (zinc, copper, magnesium, and boron) and prebiotics (inulin) interact with dietary calcium to affect bone metabolism.

1b. **Approach (from AD-416)**

Studies will utilize human subjects and animal models. Human studies will use the Mobile Nutrition Research Laboratory, the in-house Community Studies Unit, and the Metabolic Research Unit to conduct epidemiological supplementation, fortification, and controlled feeding experiments, respectively. In each case, subjects will be fed diets containing marginal to high amounts of mineral elements to determine how specific minerals, and interactions among them, affect bone structure (as determined by light microscopy, biomechanical assessment, and densiotometry) and biomarkers [urinary deoxypyridinoline, hemoglobin A1c, and C-reactive protein]). When relevant, the modifying influence of selected hormonal (e.g., estrogen deficiency) or diet compositional (e.g., inulin) factors will be examined.

2. **Milestones for FY2009**

1. Complete data analyses on community-based trial for acid-base balance and calcium retention.
Milestone Fully Met

2. Complete data collection on a three-year study on bone health and copper and zinc supplementation.
Milestone Fully Met

3. Report whether zinc inhibits osteoclastogenesis through down-regulation of Zip1 transporter.
Milestone Substantially Met

4. Report growth phase and post growth phase of boron essentiality studies.
Milestone Substantially Met

5. Complete post-growth phase of boron essentiality study.
Milestone Fully Met

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2009

6. Report on study on whether dietary boron improves calcium absorption.
Milestone Fully Met

7. Report whether polyphenols in beans slow age-related bone loss.
Milestone Fully Met

3. **Progress Report**

To determine whether a diet high in meat protein coupled with high acid load influences bone health, calcium balance and markers of bone metabolism in 16 healthy post-menopausal women were measured in a 15-wk controlled diet study. The dietary intervention and biochemical analyses are completed and a draft manuscript is under internal review.

The long-term study to determine whether supplemental intakes of copper and/or zinc enhance the ability of calcium to attenuate bone loss in healthy postmenopausal women in a sustained manner was completed. Data obtained are currently being analyzed. Preliminary analysis indicate that supplements of 2.0 mg copper and 12.0 mg zinc per day did not markedly affect biochemical indicators of bone turnover.

To determine the function of zinc and zinc transporter, ZIP1, in the osteoclastogenesis process, a cell culture model of bone-resorbing cells (osteoclasts) was used to measure osteoclast activity and expression of ZIP1 during zinc depletion or supplementation. Analyses of cell proliferation, apoptosis, osteoclast formation, and gene expression were completed successfully.

To determine whether consumption of a diet containing adequate boron during pregnancy is important in embryological development and growth of bones, late gestation rat fetuses were taken from mothers fed low or adequate amounts of boron and analyzed for bone structure development. The dietary intervention and analyses of bone structure were completed successfully.

To determine whether natural substances in beans slow age-related bone loss, mice were fed bean hull extract supplements containing high amount of polyphenols for 3 or 6 months. Bone structure was evaluated with micro-computed tomography and bone marrow cells were extracted and cultured to determine the effect of the treatment on the function of osteoblasts and osteoclasts. The studies and biochemical analyses were completed successfully.

To determine whether dietary fatty acid composition affects variables associated with the inflammatory response and bone metabolism induced by a marginal magnesium deficiency; rats were fed a diet containing 50% of their estimated magnesium requirement and dietary fat as safflower oil or fish oil for 13 weeks. The dietary intervention and biochemical analyses were completed successfully.

To confirm that dietary fatty acid composition affects the response to a marginal magnesium deficiency, which changes over time; rats were fed a diet containing 50% of their estimated magnesium requirement and dietary fat as sunflower oil, canola oil, or 50:50 sunflower oil and fish oil for and examined at 8, 16 and 24 weeks. The dietary intervention and biochemical analyses for the 8 and 16 week periods were completed.

NP / Component Coding

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4. **Accomplishments**

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01 High meat protein diet with high potential acid load does not impair calcium retention. Whether consuming meat protein is good or bad for calcium nutrition and bone health is controversial. The protein effect on bone is further compounded by a greater renal acid load when emphasizing grain-based foods rather than fruits and vegetables. ARS scientists at Grand Forks, ND, measured dietary calcium retention in healthy post-menopausal women consuming diets that were low in meat protein and potential acid load or high in meat protein and acid load. The diet rich in protein from meat improved calcium absorption that compensated for increases in urinary calcium. The high meat protein diet also increased a blood hormone known to stimulate bone formation (insulin-like growth factor I, or IGF-I). These findings indicate that consumption of beef and other meats may be beneficial, rather than detrimental to bor health. These results provide evidence useful for developing dietary recommendations for meat protein intake to maintain healthy bones.

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02 Bone loss induced by calcium deficiency in young rats can be restored by subsequent adequate calcium intake. Achieving maximal bone mass in adolescence is essential to prevent age-related bone loss. Whether inadequate calcium intake before sexual maturity can be corrected by calcium repletion afterwards is not well investigated. ARS scientists at Grand Forks, ND, demonstrated that calcium deficiency caused bone loss in female rats but the loss of bone can be reversed by increasing dietary calciu intake after sexual maturity. The results indicate that adequate calcium intake early in adulthood is important to increase bone mass and correct low bone mass due to poor calcium nutrition during adolescence. These animal results are useful for recommendir human calcium requirements to prevent osteoporosis.

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03 Pinto bean hull extract supplementation favorably affects markers of bone metabolism and bone structure in mice. Dry edible beans have many health benefits due to their high content of protein, non-digestible starches, fiber, and other bioactive components. Hulls from dry edible beans are rich in phenolic compounds recognized as possessing antioxidant activity. ARS scientists at Grand Forks, ND, showed that bean hull extract supplementation at 800 mg/kg for 3 months decreased serum tartrate-resistant acid phosphatase and parathyroid hormone concentrations in mice. Bean hull extract supplementation also improved bone structural indices, bone mineral density and trabecular thickness in the third lumbar vertebra. These findings suggest that bean hull extract may have beneficial effects on bone health by decreasing bone resorption.

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5. **Significant Activities that Support Special Target Populations**

Project Number: 5450-51000-039-00D Accession: 0409965 FY: 2009

6. Technology Transfer

0 Number of New CRADAs
0 Number of Active CRADAs
0 Number of New MTAs (providing only)
0 Number of Invention Disclosures Submitted
0 Number of Patent Applications Filed
0 Number of New Germplasm Releases
0 Number of new commercial licenses granted
0 Number of web sites managed
0 Number of non-peer reviewed presentations and proceedings
0 Number of newspaper articles and other presentations for non-science audiences
0 Number of Other Technology

7. International Cooperation / Collaboration

01 ENGLAND

Extramural agreement No. 58-5450-2M-F095 (Importance of Dietary Silicon for Bone Formation and Maintenance); collaboration with scientists at King's College in London, England. Completed the final step (publication in press). This collaboration resulted in a peer-reviewed publication reporting that silicon deprivation in rats did not affect bone quality, but inhibited bone growth plate closure, and increased longitudinal growth.

02 ARGENTINA

Extramural agreement No. 58-5450-3-F094 (Histologic, Histomorphic, and Biochemical Assessment of the Possible Augmentation of Bone Growth on Dental and Bone Implants by Silicon and Boron); collaboration with scientists at the University of Buenos Aires, Argentina. The Agreement was terminated in 2009. Two publications reported that boron is required for optimal osteoblast (bone-forming cell) activity, and low boron status impairs bone healing after injury. A third manuscript is under review.

Scientific Publications: Log 115

1. Cao, J.J., Gregoire, B.R., Gao, H. 2009. High-fat Diet Decreases Cancellous Bone Mass But Has No Effect on Cortical Bone Mass in the Tibia in Mice. Bone. 44:1097-1104. 000023290

2. Hunt, C. 2008. Dietary boron: possible roles in human and animal physiology. Biomedical Research on Trace Elements. 19(3):243-253. 000023156

3. Hunt, C., Nielsen, F.H. 2009. Nutritional Aspects of Minerals in Bovine and Human Milks. In: McSweeney, P.L.H., Fox, P.F. Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Minor Constituents. 3rd Edition. New York, NY:Springer Science+Business Media, LLC. p. 391-456. 000022205

4. Jugdaohsingh, R., Calomme, M.R., Robinson, K., Nielsen, F.H., Anderson, S., D'Haese, P., Geusen, P., Loveridge, N., Thompson, R., Powell, J.J. 2008. Increased longitudinal growth in rats on a silicon-depleted diet. Bone. 43(3):596-606. 000021347

5. Nielsen, F.H. 2009. Boron Deprivation Decreases Liver S-Adenosylmethionine and Spermidine and Increases Plasma Homocysteine and Cysteine in Rats. Journal of Trace Elements in Medicine and Biology. 23:204-213. 000023283

6. Nielsen, F.H., Stoecker, B.J. 2009. Boron and Fish Oil Have Different Beneficial Effects on Strength and Trabecular Microarchitecture of Bone. Journal of Trace Elements in Medicine and Biology. 28(3):195-203. 000020936

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 09/08/2009

Project Number: 5450-51000-039-05N **Accession:** 0407993 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** FORREST H NIELSEN

Start Date: 03/01/2004 **Term Date:** 02/28/2009

National Programs: 107 N Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE HEALING AND REMODELING BY BORON

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Enhance the quality of life through establishing mineral intakes that support optimal bone and joint health. Specifically, to confirm that boron is bioactive in osteogenesis and thus promotes bone growth and remodeling; and to establish dietary recommendations for boron that promote bone repair and maintains bone health.

1b. **Approach (from AD-416)**

Studies will use a mouse model of bone repair after injury. Mice will be fed boron-deficient and adequate diets. After about 5 weeks, surgical procedures will be performed for the purpose of evaluating peri-implant bone healing of the tibia and mandibular bone remodeling upon tooth extraction. Thirty days after the surgical procedures, the tibias and mandibles will be collected for histologic and histomorphometric examination. Tissues and plasma will be collected for the determination of indicators of bone formation and remodeling.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the UNIVERSITY OF SALTA. Additional details for the research can be found in the report for the parent project 5450-51000-039-00D, MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling after tooth extraction. The experimental portion of the research has been completed. A final manuscript was prepared reporting that nutritional boron deficiency for only 14 days did not significantly decrease incisor enamel thickness and the calcium/phosphorus ratio in enamel crystals that were formed in weanling rats. These findings contrast to other findings obtained showing that boron deprivation compared to nutritional intakes of boron resulted in impaired alveolar bone formation without tooth extraction and alveolar bone healing after tooth extraction in experimental animals because of reduced osteogenesis (bone formation). However, the boron-deficient compared to boron-supplemented rats exhibited lower enamel thickness values at each of the sites examined. Because dental enamel formation cells take about 7.5 days to secrete the enamel layer and another 12-14 days for the enamel crystals to mature, 14 days may have not been enough time on the boron-deficient regimen to induce a significant difference. The findings suggest that further study is needed to determine whether extended boron

Project Number: 5450-51000-039-05N **Accession:** 0407993 **FY:** 2009

deficiency during tooth development changes the maturation of dental enamel such that it changes the shape of teeth, or makes teeth more susceptible to caries and dentine sensitivity.

The ADODR monitored research activities through email and personal contact at a scientific meeting, and participated in the writing of the publications through email.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51000-039-06S **Accession:** 0408592 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** CURTISS HUNT

Start Date: 07/16/2004 **Term Date:** 09/30/2008

National Programs: 107 N Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE PREVENTION OF DIABETES

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-4-0366

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To contribute to basic nutrition research on the metabolic roles of boron, particularly as it may affect insulin binding/function.

1b. **Approach (from AD-416)**

Nutritional requirements and dietary recommendations for the trace mineral boron will be investigated in cell culture and animal models and humans, through application of dietary boron deprivation, supplementation, and repletion. The human studies will be conducted with appropriate review and approval by the respective Institutional Review Boards used by the University and ARS.

3. **Progress Report**

This report documents research conducted under a Specific Cooperative Agreement between ARS and NORTH DAKOTA STATE UNIVERSITY. Additional details for the research can be found in the report for the parent project 5450-51000-039-00D, MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

Conducted analysis of muscle insulin receptor concentrations in an animal model to define the mechanism by which dietary boron decreased plasma insulin levels and increased insulin sensitivity. The findings were summarized in the parent project.

ADODR monitoring was conducted via phone calls, e-mails, and site visits.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2009

Project Number: 5450-51000-039-07T **Accession:** 0408848 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** FORREST H NIELSEN

Start Date: 07/01/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: EFFECT OF ARGININE SILICATE INOSITOL COMPLEX ON BONE AND JOINT HEALTH

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 04-5450-4-0415

Organization Name: NUTRITION 21, INC.

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To determine whether silicon as an arginine silicate inositol complex is useful in overcoming the lack of dietary silicon that may lead to pathological changes in bone or connective tissue including bone cartilage (e.g., osteoarthritis) and thus result in bone erosion or loss.

1b. **Approach (from AD-416)**

Dark agouti rats that are especially susceptible to chronic and severe forms of autoimmune arthritis will be injected with type II collagen to induce arthritis after being fed silicon-deficient and silicon supplemented (as arginine silicate inositol complex) for 5 weeks. The development of arthritis will be evaluated by a macroscopic scoring system and joint swelling measurements. During arthritis development urinary bone breakdown and plasma inflammatory variables will be determined. Five weeks after injection of collagen II the animals will be killed for the determination of blood and bone variables associated with arthritis development and bone loss. Bones will be examined histologically to evaluate the effect of dietary silicon on cartilage erosion and bone resorption.

3. **Progress Report**

This report documents research conducted under a Trust Agreement between ARS and NUTRITION 21, INC. Additional details for the research can be found in the report for the parent project 5450-51000-039-00D, MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

The purpose of this research is to determine whether silicon as a novel arginine silicate inositol complex or sodium metasilicate prevents any silicon deprivation-enhanced undesirable changes in markers of bone and connective tissue metabolism induced by pro-inflammatory agents. The experimental portion of the research has been completed. A manuscript was prepared (currently under peer-review) reporting that the acute inflammatory response induced two hours after injection of LPS (lipopolysaccharide, a cell wall component of gram-negative bacteria) was not significantly affected by silicon deprivation. However, silicon in liver, and silicon, iron, and zinc in femur were increased by LPS injection only in silicon-deprived rats. Silicon deprivation also increased blood monocytes and osteopontin, and decreased femur zinc in rats not injected with LPS. Changes in tissue iron and zinc occur in the inflammatory response. These responses to silicon deprivation support a previous report suggesting that silicon is beneficial through promoting the chronic-phase inflammatory

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response. Silicon may affect bone composition and structure through influencing chronic low-grade inflammation.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/28/2009

Project Number: 5450-51000-039-08T **Accession:** 0412602 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** JAY J CAO

Start Date: 10/01/2007 **Term Date:** 12/31/2008

National Programs: 107 N Human Nutrition

Title: CALCIUM RETENTION AS INFLUENCED BY BEEF AND OTHER DIETARY COMPONENTS THAT INDUCE AN ACID LOAD IN POSTMENOPAUSAL WOMEN

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-8-0404

Organization Name: NATIONAL CATTLEMEN'S BEEF ASSOCIATION

Progress and Outcomes:

1a. **Objectives (from AD-416)**

This investigation will test the following hypotheses in a 15-wk controlled diet study of healthy postmenopausal women:
1. Dietary calcium is retained as well from a diet high in meat protein and potential acid load as it is from a diet low in meat protein and potential acid load.
2. Urinary acid and calcium excretion adapt over time (within weeks) to diets with high- or low-acid loads.

1b. **Approach (from AD-416)**

General Approach - Twenty normal and healthy post-menopausal women will participate in the study. The volunteers will consume two diets, one low in protein (10% of energy), meat (20 g/d, 15 g/d as beef) and potential renal acid load (-17 mEg/d) and one high in protein (20% of energy), meat (74 g/d, 51 g/d as beef) and potential renal acid load (46 mEg/d). The diets will be consumed for 7 weeks each with a one week washout period (15 weeks total) in a randomized cross-over design. Both diets will contain approximately 700 mg calculated calcium to match the typical intakes of postmenopausal women. After 3 wk dietary equilibration (e.g., weeks 4 and 11), the entire 2-d menu of each diet will be radio-labeled with 47Ca. Calcium retention will be measured by whole body scintillation counting for 4 weeks after ingestion of the radio-labeled meals. Blood and urine samples will be collected to assess the effects of the diet on biomarkers of bone metabolism and renal adaptation to the acid-load. To screen for normal bone health, the bone mineral density, the volunteers will be measured by dual x-ray absorptiometry.

3. **Progress Report**

This report documents research conducted under a Trust Agreement between ARS and the NATIONAL CATTLEMEN'S BEEF ASSOCIATION. Additional details for the research can be found in the report for the parent project 5450-51000-039-00D, MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

To determine whether high-meat protein with high acid load influences calcium metabolism, 20 post-menopausal women consumed two experimental diets, high-meat protein (high acid load) and low-meat protein (low acid load) for 7 weeks each with one week break in a randomized crossover design. Calcium retention from two sets of two-day 47Ca-labeled meals was measured by whole body scintillation counting. The dietary

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intervention and chemical analyses are completed.

ADODR monitoring activities to evaluate research progress included electronic mail correspondence.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 09/23/2009

Project Number: 5450-51000-041-00D **Accession:** 0415292 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: DAVID M KLURFELD **Prin Invs:** WILLIAM T JOHNSON

Start Date: 01/23/2009 **Term Date:** 01/22/2014

National Programs: 107 N Human Nutrition

Title: MITOCHONDRIAL FUNCTION AND NUTRITIONAL PROGRAMMING IN THE PREVENTION OF DIET-RELATED DISEASE

Period Covered **From:** 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Overall, to determine, using animal models, whether iron (Fe), zinc (Zn), copper (Cu), or protein intakes optimize mitochondrial function either directly or through nutritional programming during early development to facilitate the prevention of diet-related diseases, with major emphasis on cardiovascular disease. Specific objectives are: (1) determine whether maternal protein restriction produces epigenetic changes in offspring that result in impaired mitochondrial function, vascular function or other metabolic or physiologic parameters; (2) determine whether placental insufficiency that causes global fetal undernutrition rather than maternal undernutrition leads to epigenetic changes that compromise physiological function in later life of the offspring; (3) determine if marginal intakes of Fe or Zn cause oxidative and nitrative damage to proteins, lipids and DNA in cardiac mitochondria by overproduction of reactive oxygen species caused by perturbations in the electron transport chain; (4) determine if marginal Fe or Zn intakes promote apoptosis in cardiomyocytes; (5) determine the relationship between marginal intakes of Fe, Cu or Zn during pregnancy and lactation and age-related cardiac pathology in the first generation caused by altered expression of genes encoding proteins of the electron transport chain in cardiac mitochondria; and (6) determine whether marginal Fe, Cu or Zn intakes lead to the development of hypertension through reactive oxygen mediated vascular injury.

1b. Approach (from AD-416)

Three approaches using laboratory animals will be used: (1) to determine the influence of maternal diets on nutritional programming during early development, female rats will be fed diets containing either marginal levels of the nutrient of interest (protein, Fe, Zn or Cu) or, as control animals, normal nutrient levels throughout pregnancy and lactation; (2) to determine the effects of placental insufficiency in rats, uteroplacental perfusion pressure will be reduced by surgically restricting the blood supply to the uterus on gestational day 14; and (3) to determine the effects of low Fe and Zn intakes on cardiac mitochondrial function, weanling rats will be fed diets containing marginally deficient levels of these minerals for 6-8 weeks. Offspring of rats subjected to maternal dietary restrictions or reduced uteroplacental perfusion pressure will be tested for epigenetic changes, cardiac mitochondrial dysfunction, cardiac oxidative damage, cardiomyocyte apoptosis and altered vascular responses at various ages ranging from 21 days to 1 year. Weanling rats subjected to direct dietary treatments will be similarly tested at the end of the diet treatment period. Epigenetic changes will be assessed by determining DNA methylation and the up- and/or down-regulation of

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differentially methylated genes will be confirmed by real-time PCR. Measurements of mitochondrial function will include respiration, respiratory complex activity and composition, and reactive oxygen production. Mitochondria are a major source of reactive oxygen species and have a key role in regulating apoptosis. Assessment of the outcomes of mitochondrial dysfunction will extend to measurement of oxidative and nitrosative damage to mitochondrial proteins and DNA and of the susceptibility of cardiomyocytes to apoptosis. Blood pressure and vascular responses to constrictors and relaxants will be measured to determine the influences maternal treatments on vascular function in offspring and the direct effects of dietary treatments in weanling rats.

2. Milestones for FY2009

1. This is a new project with no milestones due in the reporting period. For the status of earlier milestones see previous project(5450-51000-038-00D).
   Milestone Fully Met

3. Progress Report

One of the objectives of the project is to determine the relationship between marginal iron intake during pregnancy and lactation and age-related cardiac pathology in the first generation caused by altered expression of genes encoding proteins of the electron transport chain in cardiac mitochondria. An experiment was initiated to accomplish this goal. Female rats were placed on low iron treatment for three weeks prior to conception. However, this experiment is ongoing and reportable results are not yet available. Several of the objectives of this project involve the role of nutritional programming in promoting disease in offspring whose mothers consumed suboptimal diets during pregnancy and lactation. Pilot studies were initiated to develop methodology that will be used in studies involving nutritional programming. Methodology was developed that focused on the use of DNA methylation microarrays. Methods involved isolation of DNA and genomic fragmentation with restriction enzymes followed by enrichment of methylated DNA by using an anti 5-methyl cytidine antibody. Further work included learning how to handle and interpret the DNA methylation microarray data. DNA methylation microarrays will be used to determine whether epigenetic events (DNA methylation) are responsible for the effects of nutritional programming.

NP / Component Coding

107	2	A	2009
107	3	A	2009
107	4	B	2009

4. Accomplishments

5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

Scientific Publications: **Log 115**

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51530-010-00D **Accession:** 0415315 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** FORREST H NIELSEN

Start Date: 01/26/2009 **Term Date:** 04/30/2010

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Improve health and enhance quality of life by determining, for healthy and at-risk populations (e.g., school-aged children, rural elderly, Native Americans), mineral intakes that promote optimal physiological and psychological development, function and health; develop new functional bases for establishing mineral element requirements; identify mechanisms of action; and determine the influence of sex, age, genetic, lifestyle and environmental factors on mineral element requirements. Develop and implement health promoting interventions for prevention of obesity and co-morbidities in American Indian population in the upper Midwest.

1b. Approach (from AD-416)

Dietary intakes and biochemical indices of mineral status will be related to physiologic (e.g., body composition, weight maintenance, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine roles of specific minerals in supporting optimal function and development. A Mobile Nutrition Research Laboratory, Community Studies Unit, and a residential Metabolic Research Unit will be used to conduct epidemiologic, supplementation, fortification, and controlled feeding studies, respectively with healthy and at-risk subjects (e.g., school-aged children, rural elderly, Native Americans). Use qualitative assessment methods (focus groups and in-depth interviews) and surveys to develop and implement social ecological, culturally-sensitive and scientifically sound interventions in American Indian communities. Randomized controlled trials will evaluate the effects of graded intakes of minerals, such as iron, zinc, copper, mangesium and boron, and mediating factors (e.g., genotype, controlled stressors). Animal studies will be used to determine the mechanisms of action of functional outcomes. Studies will involve university, industry and government collaboration.

2. Milestones for FY2009

1. Plan and train for phase one of the USDA-ARS multicenter "Healthy Eating and Lifestyle for Total Health" (HEALTH) study.
   Milestone Substantially Met

3. Progress Report

This project is a continuation of Project No: 5450-51000-009-00D and is acting as a bridging research project to the newly proposed project entitled "U.S. Dietary Guidelines Adherence and Healthy Body Weight." This project will identify barriers/facilitators to adhering to the Dietary Guidelines and determine the

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effectiveness of dietary and physical activity practices based on the dietary guidelines in preventing unhealthy weight gain and minimizing risk factors for obesity-related chronic disease.
Scientists from ARS and the coordinating Center for the HEALTH study conducted training in nominal group technique methods and completed cognitive interviews of proposed study questions as outlined in the timeline for the HEALTH study.

NP / Component Coding

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4. Accomplishments

5. Significant Activities that Support Special Target Populations

Scientists in the unit continue to work with American Indians to develop successful partnerships and to promote research on health promotion. This activity includes the continuation of a Cultural Awareness Workshop at United Tribes Community College attended by researchers, technicians, and administrators from throughout the Northern Plains Area. This activity supports Grand Forks Human Nutrition Research Center Programs to improve the nutrition and health of this at-risk and underserved population in the region, and facilitates accomplishment of the objective to promote health and prevent obesity in American Indian communities.

The Specific Cooperative Agreement (Subordinate project #58-5450-6-351 A2) to promote collaborative research partnerships with Cankdeska Cikana Community College (Spirit Lake Reservation) has been continued. This agreement seeks to formalize relationships to initiate discussion geared to develop culturally-appropriate activities and intervention to promote health and prevent obesity and diabetes among American Indians in the Northern Great Plains.

7. International Cooperation / Collaboration

Scientific Publications: **Log 115**

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51530-010-01R **Accession:** 0412541 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** HENRY C LUKASKI

Start Date: 10/01/2007 **Term Date:** 12/31/2009

National Programs: 107 N Human Nutrition

Title: EFFICACY OF ZINC SUPPLEMENTATION ON DIARRHEA INCIDENCE IN AN ADULT POPULATION IN WESTERN KENYA

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 60-5450-8-0400

Organization Name: U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

ARS will receive blood samples and measure blood biochemical indicators of iron, zinc, selenium nutritional status and indicators of inflammation of human volunteers.
ARS will ship samples for vitamin A and retinol-binding protein to Pennington Research Center where USARIEM has a contract to run their blood chemistries.
ARS will provide the results of analytical tests to US Army Research Institute of Environmental Medicine (USARIEM) co-investigators.
USARIEM will recruit human volunteers, randomize volunteers to treatment groups, and provide supplements and placebo treatments.
USARIEM will obtain, prepare and ship blood samples to ARS.
USARIEM will recruit and train field research associates who will collect data on diarrhea incidence and symptoms.
USARIEM collaborators will evaluate the effects of zinc supplementation on clinical assessments of diarrhea and some potential mediating factors.
ARS and USARIEM jointly will participate in interpretation of findings and preparation of reports and manuscripts.

1b. Approach (from AD-416)

The study will use a double-blind (observer blind and volunteer blind), randomized controlled design (randomization ratio 1:1) of supplemental zinc (20 mg/d) compared to placebo (maltodextrin) for five months. Randomization will include a total of 500 eligible adults matched by sex and age (18 to 55 years) and living in Western Kenya. This field research will be supervised and coordinated by investigators of the US Army Medical Research Unit - Kenya (USAMRU-K) and the Kenya Medical Research Institute (KEMRI)/Walter Reed Project (WRP), and conducted at the Kombewa Clinical Research Center (KCRC) outside of Kisumu in Western Kenya. This study will test the hypothesis that zinc supplementation reduces the incidence and symptoms of diarrhea and decreases presence of bacterial vectors associated with diarrhea.

3. Progress Report

This report documents research conducted under a Reimbursable Agreement between ARS and the U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE (USARIEM). Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Project Number: 5450-51530-010-01R **Accession:** 0412541 **FY:** 2009

The project constituted collaborative studies of the Grand Forks Human Nutrition Research Center and the USARIEM to determine the efficacy of zinc supplementation in the prevention of chronic diarrhea in an area where that problem is endemic. We completed the supplementation trial and analysis of blood samples for biomarkers of inflammation (C-reactive protein), iron status (serum ferritin), and other trace elements and electrolytes. We found that supplemental zinc reduced the incidence of diarrhea in Kenyan adults.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2009

Project Number: 5450-51530-010-02N **Accession:** 0409328 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** GERALD F COMBS

Start Date: 05/26/2005 **Term Date:** 12/31/2009

National Programs: 107 N Human Nutrition

Title: ASSESSMENT OF MINERAL LOSSES IN SWEAT DURING PHYSICAL ACTIVITY

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-5-0107N

Organization Name: GATORADE SPORTS SCIENCE INSTIT.

Progress and Outcomes:

1a. **Objectives (from AD-416)**

This project will evaluate the feasibility of determining sweat losses of mineral elements in humans during periods of controlled physical activity. This study is an initial effort to develop a valid and reliable method to determine surface mineral losses. This method is needed to improve assessment of mineral nutritional needs of physically active people.

1b. **Approach (from AD-416)**

The cooperator will recruit and enroll skilled athletes to participate in the approved study to determine the effects of physical activity on body sweat losses. The cooperator will provide unique sweat collection apparatus and position them on various sites of the body before controlled physical activities, monitor mineral-containing beverage intake, then remove the sweat collection devices. Sweat will be extracted and sent to the USDA, ARS Grand Forks Human Nutrition Research Center for determination of mineral concentrations. Data will be shared between ARS and cooperator scientists and prepared for publication in a scientific journal.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the GATORADE SPORTS SCIENCE INSTITUTE. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

The project constituted collaborative studies of the Grand Forks Human Nutrition Research Center and the Gatorade Sport Science Center to determine the composition of mineral nutrients in sweat. We completed the analyses of sweat and interstitial fluid samples collected from exercising volunteers, finding that sweat contains significant amounts of zinc and calcium. We found that supplemental zinc reduced the incidence of diarrhea in Kenyan adults.

ADODR monitoring included phone calls and e-mails.

NP / Component Coding

Project Number: 5450-51530-010-02N **Accession:** 0409328 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2009

Project Number: 5450-51530-010-03N **Accession:** 0411613 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** HENRY C LUKASKI

Start Date: 02/01/2007 **Term Date:** 12/31/2009

National Programs: 107 N Human Nutrition

Title: DIET AND EXERCISE ON PROTEIN EXPRESSION IN MUSCLE

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0110N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To identify micro-nutrient responsive proteins in muscle and other tissues obtained from rodent models.

1b. **Approach (from AD-416)**

Laboratory rodents of varying ages and, in some cases varying genotypes and phenotypes, will be fed diets containing micronutrients in marginally-deficient and adequate amounts and either exposed to physical training or untrained. Comparisons will be made among established biochemical and physical markers of nutritional status and expression of proteins in selected tissues to determine impacts of sub-clinical micronutrient deficiencies. Emphasis will be muscle mitochondrial complexes and signal transduction in mitogenesis and angiogenesis.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the US ARMY RES INST ENVIR MEDICINE. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

The project constituted collaborative studies of the Grand Forks Human Nutrition Research Center and the USARIEM in the broad area of interaction of diet and physical activity on expression of proteins that regulate structural and functional adaptations of skeletal muscle and other key organs. We completed analyses of samples collected in a study to determine the effect of exercise (swimming) duration on the use of fat/carbohydrate for energy production by muscle in the rat model. Our findings showed that prolonged exercise increased this control, but that this was not achieved by exercise-induced food (energy) restriction.

ADODR monitoring is done via phone calls, e-mails, conference meetings.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/18/2009

Project Number: 5450-51530-010-04T **Accession:** 0412129 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** FORREST H NIELSEN

Start Date: 05/01/2007 **Term Date:** 12/31/2009

National Programs: 107 N Human Nutrition

Title: MAGNESIUM NUTRITION AND SLEEP BEHAVIOR IN OLDER ADULTS

Period Covered **From:** 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0431

Organization Name: TYRASE RESEARCH

Progress and Outcomes:

1a. **Objectives (from AD-416)**

The objective of this cooperative research project is to determine the association between magnesium nutrition (intakes and status) and sleep behavior (quantity, quality, disturbances) in older adults; to determine the efficacy of magnesium supplementation to improve sleep (increase quantity and quality and prevent or reduce disorders); and to identify factors (for example, gender, health, diet, body composition, physical activity, depression - historical or current) that mediate or moderate the relationship between magnesium nutrition and sleep.

1b. **Approach (from AD-416)**

An experiment will be performed that will have an 8-week double-blind placebo-controlled cross-sectional design. People with sleep complaints (for example, insomnia, nighttime awakenings, difficulty in falling asleep, awakening too early, not feeling rested after sleep) will be recruited. Following baseline assessment during week one of past and current health, diet, body composition, physical activity, depression, and sleep, 100 adults (50 males and 50 females) aged older than 51 yrs will be randomly assigned to one of two groups of 50 each. Groups will be matched by gender, age and overall sleep score and magnesium status (determined by erythrocyte magnesium and calcium, and plasma total and ionized magnesium). Then one group will be given a 300 mg/day magnesium gluconate supplement for 7 weeks while the other group will be given a placebo. An assessment of health, diet, body composition, physical activity, depression, sleep and magnesium status will occur during weeks 6 and 8, concluding the study.

3. **Progress Report**

This report documents research conducted under a Trust Agreement between ARS and TYRASE RESEARCH. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

The study was completed and a final report prepared. The findings will be reported in a peer-reviewed publication. The purpose of the research was to determine whether a low magnesium status contributes to the high prevalence of sleep disturbances in older adults. Sleep quality as determined by the overall Pittsburg Sleep Quality Index was significantly improved Red blood cell magnesium also increased regardless of supplementation but the increase was about 70% greater in the magnesium-supplemented group. The reason for the overall increase is unclear, but may be related to increased citrate intake with meals. Magnesium was supplemented as magnesium citrate and the

Project Number: 5450-51530-010-04T **Accession:** 0412129 **FY:** 2009

placebo was sodium citrate. When all subjects were included in the analyses, magnesium supplementation did not significantly affect numerous biochemical variables that respond to a severe magnesium deficiency in experimental animals. However, when subgroups were examined, magnesium supplementation significantly reduced C-reactive protein (CRP) in subjects whose baseline values were higher than 3.0 (an indication of inflammatory stress). Thirty-seven subjects had baseline serum magnesium concentrations below 1.8 mg/mL, the low value for normal serum magnesium. When only these subjects were included in the analysis, bother serum magnesium and calcium significantly increased during the study with the increase more marked in the magnesium-supplemented group. The findings indicate that a significant number of individuals older than 51 years have a low magnesium status, and that magnesium supplementation may alleviate some chronic low-grade inflammation, which has been associated with poor sleep quality.

Because the study was performed in-house, the ADODR actively participated in the research by conducting information meetings, assessing the validity of the procedures followed, and addressing problems with equipment, forms and procedures occurring during the study. Progress in this project was communicated to Tyrase Research by the ADODR through telephone calls and a final report.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2009

Project Number: 5450-51530-010-07N **Accession:** 0410137 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** GERALD F COMBS

Start Date: 03/09/2006 **Term Date:** 09/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL NUTRITION RESEARCH

Period Covered **From:** 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0101N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Collaborator in planning, implementation and reporting of research on the effects of minerals on human nutritional needs and physical and psychological performance.

1b. **Approach (from AD-416)**

Human volunteers will be studied under a variety of dietary conditions and biochemical and functional parameters will be measured.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the US ARMY RES INST ENVIR MEDICINE. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

The project constituted collaborative studies of the Grand Forks Human Nutrition Research Center and the USARIEM in the broad area of nutrition research with emphasis on weight management and bone health. We developed a plan for a collaborative study to determine whether high-protein diets can be effective in mitigating the decline in resting energy expenditure and the losses of lean body mass and bone mineral density typically attending weight reduction caused by energy imbalance. This human study will be conducted at the GFHNRC in FY2010.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/18/2009

Project Number: 5450-51530-010-08S **Accession:** 0411236 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** GERALD F COMBS

Start Date: 09/19/2006 **Term Date:** 08/31/2011

National Programs: 107 N Human Nutrition

Title: HEALTH PROMOTION IN AMERICAN INDIAN COMMUNITIES

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0351

Organization Name: CANKDESKA CIKANA COMM COLLEGE

Progress and Outcomes:

1a. Objectives (from AD-416)

The broad objective of this cooperative research is to develop information useful in promoting health through improved nutrition and lifestyles. The specific objectives are to:
1. Develop understanding of the relationships of diet, lifestyle, and the prevalence of chronic diseases, particularly obesity, diabetes, and cardiovascular disease in American Indian peoples;
2. Identify health needs, and the barriers to and facilitators of meeting those needs in American Indian communities;
3. Determine the efficacy of community-based, health-promoting, intervention strategies in American Indian communities; and,
4. Increase research cooperation between American Indian colleges and USDA-ARS.

1b. Approach (from AD-416)

Identification and characterization of barriers to and facilitators of eating healthy diets and engaging in healthy lifestyles will be accomplished through a series of focus groups in American Indian communities. Focus groups will be designed to also identify and prioritize community needs as potential mediating factors. Subsequently, health-promoting intervention strategies will be developed and evaluated based on identified barriers and facilitators in the context of community needs and priorities. A research involving human subjects will be conducted with appropriate review and approval by the respective institutional review boards used by Cankdeska Cikana Community College and ARS.

3. Progress Report

This report documents research conducted under a Specific Cooperative Agreement between ARS and the CANKDESKA CIKANA COMMUNITY COLLEGE. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

We collaborated with the Cankdeska Cikana Community College of the Spirit Lake Dakota Nation to continue data analysis of a community needs assessment, and to plan an ancillary study of the multi-center, ARS HEALTH study for implementation in FY2010.

ADODR monitoring includes site visits, meetings, phone calls, and e-mails.

NP / Component Coding

Project Number: 5450-51530-010-08S **Accession:** 0411236 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2009

Project Number: 5450-51530-010-09N **Accession:** 0412737 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** GERALD F COMBS

Start Date: 01/01/2008 **Term Date:** 12/31/2012

National Programs: 107 N Human Nutrition

Title: GRAND FORKS COMMUNITY-BASED HEALTH AND FITNESS AGENDA

Period Covered **From:** 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-8-0101N

Organization Name: GRAND FORKS PARK DISTRICT

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To develop a long-term partnership to foster the development of effective wellness/fitness programs in the Greater Grand Forks Community that will provide opportunities for community based research addressing issues related to the prevention of obesity.

1b. **Approach (from AD-416)**

The Grand Forks Parks District (GFPD) will work with community groups to develop wellness/fitness programs and facilities in the Greater Grand Forks Community. The Grand Forks Human Nutrition Research Center (GFHNRC) will work with the GFPD to advise on issues related to the health needs of citizens, and the design and implementation of those programs/facilities. Both institutions will work together to identify strategic linkages that will meet the goals of the GFPD and advance the research mission of the GFHNRC.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the GRAND FORKS PARK DISTRICT. Additional details for the research can be found in the report for the parent project 5450-51530-010-00D, MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

We collaborated with the Grand Forks Park District in a summer parks based program for 300 elementary schoolers, in which we used sessions emphasizing personal hygiene and healthy food choices, to determine the feasibility of using pedometers to assess unmonitored physical activity in this age group. We found subjects in this age group eager to learn about healthy foods when presented in a hands-on, "build your own healthy snack" modality.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/17/2009

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-042-00D **Accession:** 0415303 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Prin Invs:** LIN YAN

Start Date: 01/15/2009 **Term Date:** 04/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered **From:** 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. **Objectives (from AD-416)**

The general objective is to determine how current and proposed changes to the American diet that may adversely affect intake and/or how bioavailability of the essential mineral nutrients can be modified to enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Specific objectives are:

Objective 1: Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. This objective will address the production of foods with enhanced bioactive Se compounds, and assess their ability to enhance health, especially by controlling oxidative stress and reducing cancer risk. The impact of organic farming methods will also be assessed (Finley). It will also address the practical impact of dietary changes that emphasize plant-based diets on meeting nutritional needs for Fe and Zn (Hunt).

Objective 2: Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. This objective will evaluate the bioavailability of Fe fortificants such as elemental Fe and micronized, encapsulated Fe compounds in human studies (Hunt).

Objective 3: Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components. Cell and whole animal models will be employed to elucidate how the modifications of mineral content of foods can influence the biochemical regulation of specific transporters, cellular trafficking, and interactions of minerals such as Zn, Fe, Cu, Cd, Se, and Mn. (Reeves).

Problem to be addressed with increased funds: Elucidate the roles and diets in support of optimal health and prevention of obesity and related illnesses, cardiovascular disease, osteoporosis and cancer.

Problem to be addressed with increased funds (FY05): Under Performance Measure 4.1.1 of the ARS Strategic Plan and the NP107 Action Plan, this project will develop an enhancement to the food supply by increasing the nutritional value of beef.

Project Number: 5450-51000-042-00D **Accession:** 0415303 **FY:** 2009

Objective modification FY05: Increase the amount of omega-3 fatty acids in beef to a nutritionally significant level by feeding flax. Demonstrate that the increase in omega-3 fatty acids in the meat are sufficient to have a physiological effect. Study feasibility of increasing selenium in beef to levels that will have an impact on human health when the meat is consumed at recommended levels. This may include studies of organic form of selenium in beef, stability with varying cooking methods, sensory issues, bioavailability and health effects in both steers and consumers.

1b. Approach (from AD-416)

Methodology will include tests of agricultural conditions affecting the amounts and forms of minerals incorporated into in foods; in vitro, cellular, and animal models of mineral transport and absorption; and human experiments with controlled diets to assess mineral absorption, retention, and biological function and to model nutritional requirements.

Specific objectives to be accomplished with increased funding: To study the roles of foods, particularly those produced in the Northern Plains, in the support of health. This work is to be multi-disciplinary, including collaborations such as with the University of North Dakota School of Medicine and Health Sciences and North Dakota State University.

2. Milestones for FY2009

1. Report high-Se soy protein bioavailability study.
   Milestone Fully Met

3. Progress Report

This project is a bridging project that continues the work of Project No: 5450-51000-035-00D. During the course of this project, we completed studies that assessed bioavailability of selenium from high-selenium peas and oats produced in South Dakota. We found that selenium from these peas and oats were highly bioavailable to selenium-deficient animals. These results may lead to further studies assessing the nutritional values and health benefits of this natural dietary source of selenium, and they will also increase the marketability of agricultural products from the high-selenium Northern Plains, especially to selenium-deficient countries. This research will not be continued because of the Center's re-direction to obesity-related research. However, some results from this project (e.g. high-selenium crops produced in Northern Plains) will be useful for a new project "Dietary Modulation of Obesity-Related Cancer by Selenium".

NP / Component Coding

107	1	C	2009

4. Accomplishments

01 Selenium from high-Se peas and oats produced in Northern Plains is bioavailable. Selenium is an essential mineral nutrient to humans. Northern Plains are rich of selenium in its soil, and agricultural produces from Northern Plain are high in this nutrient. ARS scientists in Grand Forks, ND assessed bioavailability of selenium from high-selenium peas and oats produced in South Dakota. Selenium from these products wa highly bioavailable to selenium-deficient rats compared with selenomethionine, a food form of selenium. These results may lead to further studies assessing the nutritional values and health benefits of this natural dietary source of selenium, and these

Project Number: 5450-51000-042-00D Accession: 0415303 FY: 2009

results will increase the marketability of agricultural products from Northern Plains

107 1 C 2009

5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

Scientific Publications: Log 115

Approved: MCGUIRE MICHAEL R Date: 09/02/2009

Project Number: 5450-51000-042-01T **Accession:** 0408646 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Prin Invs:** JAY J CAO

Start Date: 05/01/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM PINTO BEANS AS A VALUE-ADDED PRODUCT

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-4-0418

Organization Name: NORTHARVEST BEAN GROWERS ASSOCIATION

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Determine the selenium content of pinto beans. Determine factors that increase the concentration of selenium in pinto beans. Use GIS data base to model mineral accumulation in pinto beans.

1b. **Approach (from AD-416)**

Pinto bean samples will be obtained at the time of harvest directly from producers in central North Dakota. Beans will be analyzed for minerals including selenium. Data on mineral content will be combined with producer information and data from the GIS data base. These data will be modeled by statistical methods to determine factors that result in selenium accumulation in pinto beans.

3. **Progress Report**

This report documents research conducted under a Trust Agreement between ARS and the NORTHARVEST BEAN GROWERS ASSOCIATION. Additional details for the research can be found in the report for the parent project 5450-51000-042-00D, MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

The purpose of this agreemént was to determine factors that affect the accumulation of selenium in pinto beans. Dry edible beans are a major crop raised in North Dakota, and there may be substantial health benefits associated with consumption of beans. With a change in personnel on this project, studies were re-designed and initiated to evaluate the effects of selenium and other antioxidants in beans on bone metabolism in a mouse model and on the cellular functions of bone-forming (osteoblasts) and bone-remodeling (osteoclasts) cells in a cell culture system.

An experiment has been performed to determine whether selenium from pinto beans is bioavailable to support bone health and functions of osteoclasts and osteoblasts. Another experiment has been conducted to determine whether bean hull extract supplementation affects bone structure of femurs and osteoblast function.

ADODR monitoring activities to evaluate and discuss the research progress included teleconferences and electronic mail correspondence.

NP / Component Coding

Project Number: 5450-51000-042-01T **Accession:** 0408646 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51000-042-02N **Accession:** 0411932 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Prin Invs:** GERALD F COMBS

Start Date: 01/01/2008 **Term Date:** 12/31/2012

National Programs: 107 N Human Nutrition

Title: COLLABORATION TO PROMOTE RESEARCH ON HEALTH ROLES OF WHOLE GRAINS

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-8-0103N

Organization Name: UNIVERSITY OF MINNESOTA

Progress and Outcomes:

1a. Objectives (from AD-416)

This non-funded cooperative agreement (NFCA) is to establish a general framework for cooperation and coordination between the Grand Forks Human Nutrition Research Center (GFHNRC) of the USDA, ARS, and the University of Minnesota Institute for Grains and Health Research (IGHR). The NFCA establishes a mechanism whereby the GFHNRC and IGHR will pursue active and fruitful scientific collaborations relating to the research to elucidate the health roles of grains in diets in all appropriate venues, as consistent with their respective missions and policies.

1b. Approach (from AD-416)

1. Pursue food-based human nutrition research including research addressing issues related to the health roles of grains and grain-based foods.

2. Collaborate with members of the IGHR in such research.

3. Work with the IGHR in the development of a systematic approach to elucidating the health roles of grains and grain-based foods.

3. Progress Report

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the UNIVERSITY OF MINNESOTA. Additional details for the research can be found in the report for the parent project 5450-51000-042-00D, MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

We participated in the scientific advisory group of the Grains for Health Program of the University of Minnesota, which developed three platforms (Applied Science, Child Nutrition and Sustainable Training), each contributing to the overall goal of impacting public health by improving the grain food supply at different levels of influence. Discussions involved the identification of critical gaps in knowledge regarding the each platform.

ADODR monitoring includes phone calls, e-mails, and discussions at professional meetings.

NP / Component Coding

Project Number: 5450-51000-042-02N **Accession:** 0411932 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 08/17/2009

Project Number: 5450-51000-044-00D **Accession:** 0415317 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD **Prin Invs:** ERIC O UTHUS

Start Date: 01/26/2009 **Term Date:** 04/30/2010

National Programs: 107 N Human Nutrition

Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Determine the molecular and cellular mechanism(s) of action of selenium (Se) in anti-carcinogenesis. Specific objectives include 1) Determine the role of Se in cell cycle progression and apoptosis in models of colon cancer; 2) Determine the role of selenoproteins in cancer prevention and the role of dietary components in the regulation of selenoprotein activity; 3) Determine the mechanism(s) by which Se alters DNA methylation and 4) Determine the relationship of oral selenium intake with selenium status and indicators of cancer risk.

1b. Approach (from AD-416)

A variety of cell culture and animal model approaches will be used. In general, cell culture experiments will be run using cell lines specific for colon. Various forms and concentrations of selenium will be added to serum-free media. Cell growth, indices of selenium status, and indices of cell cycle progression and apoptosis will be measured. These studies will be used to determine the effects of nutritional levels of selenium in supporting cellular survival signaling in human cultured colon cells, and the role of the putative anti-tumorigenic selenium-metabolite, methylselenol, in cell cycle progression and apoptosis in human cultured colon cells. Other cell culture models (colon and/or liver cell lines) will be used in siRNA knockdown studies. These experiments will determine the effect of selenium in cells in which specific genes have been knocked down by siRNA. Other studies will use knock downs of various selenoproteins to determine their role in anticarcinogenicity of selenium. Animal studies will use rats and mice to determine the effects of form and concentration of dietary selenium on 1) selenoprotein expression and activity as related to carcinogenesis, 2) carcinogen-induced aberrant crypt formation (preneoplastic colon cells) and, 3) indices of oxidative stress and one-carbon metabolism including DNA methylation of genomic and gene specific DNA.

2. Milestones for FY2009

1. Complete aberrant crypt study and related methionine sulfoxide studies.
   Milestone Fully Met

2. Develop an animal model of physical activity that is useful in obesity-related cancer research.
   Milestone Fully Met

3. Develop a rapid analytical method for selenium in foods and biological specimens.
   Milestone Fully Met

Project Number: 5450-51000-044-00D **Accession:** 0415317 **FY:** 2009

4. Develop multi-compartment model for assessing selenium status applicable to (non-deficient) healthy Americans.
Milestone Substantially Met

3. **Progress Report**
This bridging project continues the work of Project No. 5450-51000-036-00D until the new project entitled "Dietary Modulation of Obesity-Related Cancer by Selenium" is certified. Because the new project focuses on obesity-related cancer, our work has centered around that. For example, we have continued the development of animal models that can be used to show that dietary obesity enhances secondary tumor development and tumor growth. Development of these animal models will be crucial for studies on dietary intervention and prevention of obesity-related secondary cancer. A typical part of the Western diet is a high fat intake that leads to increased levels of fecal bile acids (primarily, deoxycholic acid), which are believed to promote colon carcinogenesis. It is also known that colon tumor development is driven by the accumulation of dysregulation in cellular MAPK/APC cellular signaling and the alteration epigenetic status (e.g., p53 gene and global DNA methylation). Cell models that we have developed will be used to determine whether methylselenol antagonizes the effect on cellular MAPK/APC signaling and epigenetic status caused by deoxycholic acid. We also developed a voluntary physical activity model for the mouse. This model will be useful in investigations of physical activity and/or its combination with dietary modifications on obesity-related cancer development and growth. We developed a rapid, sensitive, non-destructive analytical method for selenium using low-angle, X-ray fluorescence spectroscopy. We have optimized the 15-min method for application in foods (small grains), fluids (plasma) and cells (adipocytes). Finally, we developed a comprehensive method for assessing selenium status in non-deficient subjects using three analytical approaches (total selenium by electrothermal atomic absorption spectrophotometry, glutathione peroxidase activity by UV-spectrophotometry, and selenoprotein P by immunoassay) and imputation of the variable non-specific selenium fraction.

NP / Component Coding

107	2	A	2009
107	3	A	2009

4. **Accomplishments**

01 A voluntary physical activity model that is useful in research of obesity-related cancer: Emerging evidence in recent years shows that physical activity may protect against the occurrence of certain types of cancers. ARS scientists in Grand Forks, ND developed a voluntary physical activity model (an in-cage running wheel model) in mice, and found that the physical activity at the voluntary level significantly reduced abdominal adipose deposition and increased oxidative enzyme activities. This model will be useful in investigations of physical activity and/or its combination with dietary modifications on obesity-related cancer development and growth.

107 2 A 2009

02 ARS scientists in Grand Forks, ND developed a rapid, sensitive, non-destructive analytical method for selenium using low-angle, X-ray fluorescence spectroscopy. This method, including sample preparation, yields results within 15-min. The method has been optimized for applications in small grains, plasma and adipocytes.

107 2 A 2009

03 ARS scientists in Grand Forks, ND developed a multi-compartment model for assessing selenium status applicable to (non-deficient) healthy Americans. The method employs

Project Number: 5450-51000-044-00D **Accession:** 0415317 **FY:** 2009

multiple analytical approaches: total selenium by electrothermal atomic absorption spectrophotometry, glutathione peroxidase activity by UV-spectrophotometry, and selenoprotein P by immunoassay. With those results scientists can impute the non-specific selenium fraction variations that are found to comprise virtually all of the inter-individual variation in plasma selenium status in healthy subjects.

107 2 A 2009

5. Significant Activities that Support Special Target Populations

We found that women regulate selenium metabolism somewhat differently than men. When consuming comparable amounts of dietary selenomethionine, women retain less than men, which we believe may reflect an increased turnover rate compared to men.

7. International Cooperation / Collaboration

01 UNITED KINGDOM

United Kingdom - We collaborated with a scientist at the University of Surrey, Surrey, UK, to provide critical comment on a major clinical trial that was published recently. No agreement involved.

Scientific Publications: **Log 115**

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51000-044-01R **Accession:** 0410110 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD **Prin Invs:** GERALD F COMBS

Start Date: 09/01/2005 **Term Date:** 08/31/2010

National Programs: 107 N Human Nutrition

Title: SELENIUM NUTRITION IN HUMANS: PREDICTING DIETARY SELENIUM NEEDS TO ACHIEVE TARGET BLOOD SELENIUM LEVELS

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 60-5450-5-0330

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Develop algorithm relating increase in stable plasma Se level to that at baseline and level of supplemental Se.

1b. **Approach (from AD-416)**

Conduct a randomized, double-blind, intervention study will be conducted with healthy men (120) and women (120) randomized to 0, 50, 100, or 200 ug Se/day (as L-selenomethionine) administered in daily oral doses. Fasting blood samples and urine samples will be drawn two wks prior to and periodically throughout the 1-yr study. The following measurements will be made: Se, homocysteine, vitamin B12 and folate in plasma; Se and 8a-deoxyquanosine in urine; DNA damage and allelic variants of Se-dependent enzymes in lymphocytes. Results will be used to compute the relationship of final-plateau plasma (9-12 mos.) Se concentration as a function of baseline (0 mos.) Se level, Se dose, metabolic body size and urinary Se, as well as outcomes related to carcinogenesis.

3. **Progress Report**

This report documents research conducted under a Reimbursable Agreement between ARS and the NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH. Additional details for the research can be found in the report for the parent project 5450-51000-044-00D, ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

We developed a comprehensive method for assessing selenium status in non-deficient subjects using three analytical approaches (total selenium by electrothermal atomic absorption spectrophotometry, glutathione peroxidase activity by UV-spectrophotometry, and selenoprotein P by immunoassay) and imputation of the variable non-specific selenium fraction. Using this approach, we found that healthy American adults respond to nutritional supplements of selenium by increasing their non-specific fraction of plasma selenium, which we are presently undertaking to characterize. It appears to consist mostly of protein-bound selenomethionine plus a small proportion of bioactive selenium metabolites.

NP / Component Coding

Project Number: 5450-51000-044-01R **Accession:** 0410110 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 08/18/2009

Project Number: 5450-51000-044-02S **Accession:** 0414720 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD **Prin Invs:** GERALD F COMBS

Start Date: 09/29/2008 **Term Date:** 09/28/2011

National Programs: 107 N Human Nutrition

Title: FOOD-BASED OBESITY PREVENTION AND HEALTH MAINTENANCE RESEARCH

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-8-0342

Organization Name: UNIVERSITY OF NORTH DAKOTA

Progress and Outcomes:

1a. **Objectives (from AD-416)**

The objective of this cooperative research is to investigate the role of foods and their components in human health, with particular focus on the prevention of obesity, including the endogenous (biological) and exogenous (psycho-social, environmental) factors that affect the maintenance of healthy body weight and risk to co-morbidities of obesity.

1b. **Approach (from AD-416)**

Conduct studies with human volunteers to elucidate functions of and quantitative needs for nutrients and/or other components of foods and physical activity in the support of healthy body weight and minimization of risk to chronic disease. Includes focus groups, cross-sectional and clinical intervention studies in both residential and non-residential settings involving volunteers recruited from Grand Forks and other communities.

3. **Progress Report**

This report documents research conducted under a Specific Cooperative Agreement between ARS and the UNIVERSITY OF NORTH DAKOTA. Additional details for the research can be found in the report for the parent project 5450-51000-044-00D, ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

The project involved the initiation of one new study and the analysis of samples/data collected by nine studies the human subjects intervention components of which were previously completed.

We found dietary selenomethionine increases biomarkers of selenium status, but that this effect is dependent on genotype with respect to two selenoenzymes, glutathione peroxidase (GPX1) and selenoprotein P (SePP1). GPX1 genotype is a determinant of baseline plasma selenium level, and SePP1 genotype is a determinant of selenium balance and urinary selenium losses. IMPACT: This finding relates the metabolism of a nutrient (selenium) show capable of reducing cancer risk to a GPX1 genotype associated with increased (lung) cancer risk.

We found a high meat-protein diet with high potential acid load does not impair calcium retention in women. IMPACT: This study confirms previous work at the Center; it shows that diets high in meat protein do not impair the use of dietary calcium in healthy adults and refutes previous results with purified proteins in rats.

Project Number: 5450-51000-044-02S **Accession:** 0414720 **FY:** 2009

We conducted a randomized clinical trial to determine whether low magnesium status contributes to the high prevalence of sleep disturbances in older adults. We randomized subject (>51 y) to a supplemented of magnesium citrate or a placebo of sodium citrate. We found sleep quality (Pittsburg Sleep Quality Index) and red blood cell magnesium to increase in both groups, with the latter increase 70% greater in the magnesium-supplemented group. Magnesium supplementation significantly reduced C-reactive protein (CRP) in subjects whose baseline values were higher than 3.0 (indicating inflammatory stress). Subjects with baseline serum magnesium concentrations <1.8 mg/mL responded to magnesium supplementation with increases in both serum magnesium and calcium. The findings indicate that a significant number of older individuals are of low magnesium status, and that magnesium supplementation may alleviate some chronic low-grade inflammation, which has been associated with poor sleep quality.

Because the study was performed in-house, the ADODR actively participated in the research by conducting information meetings, assessing the validity of the procedures followed, and addressing problems with equipment, forms and procedures occurring during the study. Progress completed.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2009

Project Number: 5450-51000-044-03N **Accession:** 0412134 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD **Prin Invs:** LIN YAN

Start Date: 08/01/2007 **Term Date:** 12/31/2011

National Programs: 107 N Human Nutrition

Title: ANTICANCER EFFECTS OF HIGH-SELENIUM SOYBEANS

Period Covered From: 10 / 2008 **To:** 9 / 2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0119N

Organization Name: UNIVERSITY OF NEBRASKA

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To investigate whether high-selenium soybeans have greater anticancer effects that low-selenium soybeans in animal models.

1b. **Approach (from AD-416)**

UNL will be responsible for identifying an appropriate line of soybeans (based on its nutrient contents) and determining the stages of the plant development that are most appropriate for selenium fertilization. UNL will be responsible for planting, fertilizing the plants with selenium, harvesting and providing the beans to GFHNRC.

GFHNRC will be responsible for designing and conducting animal studies. This includes dietary preparation, feeding animals, carcinogen treatment, monitoring the progress, collecting and analyzing data.

GFHNRC and UNL together will interpret data, draw conclusions from the investigation and prepare manuscripts for publication in scientific journals.

3. **Progress Report**

This report documents research conducted under a Non Funded Cooperative Agreement between ARS and the UNIVERSITY OF NEBRASKA. Additional details for the research can be found in the report for the parent project 5450-51000-044-00D, ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

We quantified selenium from soybeans harvested from 2008 crop year, and we found the content did not reach the targeted level for planned research use. To ensure to have the soybeans with targeted selenium level, we planted two different soy cultivars in Agronomy Research Farm at University of Nebraska-Lincoln in 2009 crop year. Upon harvest in October, we will have the seeds processed to soy protein isolate for research use if they reach the targeted selenium level.

ADODR monitoring activities included phone calls, e-mails, conference calls and meetings at scientific venues.

NP / Component Coding

Project Number: 5450-51000-044-03N **Accession:** 0412134 **FY:** 2009

Approved: MCGUIRE MICHAEL R **Date:** 08/18/2009

FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: DAVID M KLURFELD **Prin Invs:** WILLIAM T JOHNSON

Start Date: 07/21/2004 **Term Date:** 01/22/2009

National Programs: 107 N Human Nutrition

Title: DIETARY COPPER REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH

Period Covered From: 10/ 2008 **To:** 9 / 2009 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Overall, to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. To develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans. To determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs. To determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation. To determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis. To determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

1b. Approach (from AD-416)

Laboratory animals of varying ages and, in some cases, varying genetic makeup will be fed diets containing copper in severely deficient, marginally deficient or adequate amounts for varying periods of time. Based on comparisons to reliable invasive markers of copper status, non-invasive biomarkers will be tested for validity in assessing marginal copper deficiency. Tests of cardiovascular function and examinations of mechanism of depressed function will be made over ranges of copper status varying from severely-deficient to adequate. Cardiovascular functional measurements include heart contractile function, cardiac mitochondrial respiration and respiratory complex activity, blood vessel contractility and blood pressure. Atherosclerosis will be assessed by microscopic observation of blood vessels. Examination of mechanisms of depressed function will focus on the effects of oxidative stress and altered nitric oxide metabolism known to occur in copper deficiency. Such examination will include assessment of oxidative/nitrosative damage, altered nitric oxide signaling and altered homocysteine metabolism and will extend to measurement of transcription factors, mRNA and enzymes that influence and are influenced by oxidative stress and nitric oxide signaling.

2. Milestones for FY2009

1. Correlate organ Cu content with combinations of indicators of Cu Status: select best combination of indicators as biomarker.
   Milestone Fully Met

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2009

2. Identify oxidized, nitrated mitochondrial proteins in Cu deficiency.
   Milestone Substantially Met
   Oxidized mitochondrial proteins have been identified but no changes in nitrated proteins were detected in heart mitochondria were detected. A manuscript describing the results is in preparation.
3. Identify mtDNA mutations of Cu deficiency.
   Milestone Not Met
   Other (a reason for not meeting the Milestone other than the ones above
   Technical difficulties were encountered in developing the protocols for measuring mtDNA mutations. These difficulties prevented completion of the milestone as scheduled.
4. Relate mtDNA mutations to expression of respiratory complexes.
   Milestone Not Met
   Other (a reason for not meeting the Milestone other than the ones above
   Technical difficulties were encountered in developing the protocols for measuring mtDNA mutations. Since mtDNA mutations could not be measured, their effect on the expression of respiratory complexes could not be assessed.
5. Does iNOS precondition Cu-deficient heart? (Hypothesis 1e contingency).
   Milestone Not Met
   Critical vacany (quantitative or qualitative deficiency in personnel

3. Progress Report

During the 5 years of this project, progress was made in identifying mechanisms for the effects of Cu deficiency on cardiovascular function and identifying low copper intake during pregnancy as a factor for cardiovascular disease in offspring. Research findings showed that hearts of copper-deficient mice have reduced contractile pressure, elevated relaxation pressure, impaired responsiveness to adrenalin excitation, and increased collagen deposition. These changes in the heart are characteristic of heart failure and show that dietary Cu deficiency is a risk factor for heart disease and subsequent cardiac failure.Furthermore, characteristics of heart failure were found in adult rats even at marginally low Cu intake. In terms of vascular function, findings from this project showed that increased blood pressure caused by copper deficiency in rats was caused by the attenuation of vascular relaxation resulting from decreased levels of the vascular relaxant, nitric oxide. It was also shown that copper deficiency promotes vascular inflammation independently of its effect on nitric oxide levels. These findings indicate that low dietary copper intake increases the risk for cardiovascular disease by promoting hypertension and vascular inflammation. Other studies performed in this project indicate that dietary copper may be important for reversing some forms of heart disease. In mice, surgical constriction of the aorta produces chronic pressure overload and eventual cardiac enlargement and failure. It was found that that copper supplementation of the mice having constricted aortas reversed the cardiac enlargement and prevented heart failure by promoting the formation of blood vessels in the heart. These findings indicate that copper requirements may increase in patients with certain types of heart disease associated with cardiac enlargement and that copper supplementation may be beneficial for improving their cardiac function. Studies with pregnant rats showed that low copper intakes during pregnancy affects cardiac health in offspring. When rats have marginally low copper intake during pregnancy and lactation, their adult, copper-repleted offspring exhibit increased heart mitochondrial hydrogen peroxide production and abnormally low activity of a copper-dependent enzyme, cytochrome c oxidase, that is important for cardiac mitochondrial energy production. Furthermore, the vascular relaxation response was altered in both the first and second generation offspring of the marginally copper deficient dams. These findings suggest that low copper intake by pregnant women may increase the risk for cardiovascular disease in their children and grandchildren. Mitochondrial dysfunction is a component

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2009

in the development of several conditions such as heart disease, diabetes and obesity. Research into the role of sub-optimal intakes of protein, iron or zinc during pregnancy in promoting disease in offspring by adversely programming mitochondrial function during early development will continue in a new project titled "Mitochondrial Function and Nutritional Programming in the Prevention of Diet-Related Disease" (5450-51000-041-00D).

NP / Component Coding

107	2	A	2009
107	4	B	2009

4. Accomplishments

01 Copper deficiency increases the risk for cardiovascular disease by promoting vascular inflammation: Experiments with laboratory animals have shown that low dietary copper intake promotes inflammation. A potential mechanism underlying the promotion of inflammation by low copper intake is the reduction of nitric oxide (NO) levels caused by the accumulation of reactive oxygen species (ROS) that react with NO to form peroxynitrite. Reduction in NO levels can increase the amount of an enzyme called cyclooxygenase (COX) that synthesizes chemicals involved in producing inflammation in blood vessels. The present study showed that low copper intake increases COX in the aorta of rats. It was also shown that the increase in COX was accompanied by increase production of chemicals that produce inflammation. However, inhibition of ROS production in the aortas of the copper-deficient rats did not lower COX or the production of inflammatory chemicals. This indicates that inflammation in the aorta caused by low copper intake is independent of ROS production and NO levels. IMPACT: This study shows that that low copper intake can increase the risk for cardiovascular disease by increasing the production of pro-inflammatory chemicals in blood vessels.

107 2 A 2009

5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

Scientific Publications: **Log 115**

1. Falcone, J.C., Lominadze, D., Johnson, W.T., Schuschke, D.A. 2008. Endothelial Cell-Derived Nitric Oxide Mobilization is Attenuated in Copper-Deficient Rats. Applied Physiology, Nutrition & Metabolism. 33:1073-1078. **000024315**
2. Nielsen, F.H. 2009. Marginal Zinc Deficiency Increases Magnesium Retention and Impairs Calcium Utilization in Rats. Biological Trace Element Research. 128(3):220-231. **000023283**
3. Zhou, Z., Johnson, W.T., Kang, Y.J. 2009. Regression of Copper-Deficient Heart Hypertrophy: Reduction in the Size of Hypertrophic Cardiomyocytes. Journal of Nutritional Biochemistry. 20(8):621-628. **000024314**

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2009

ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Prin Invs:** HENRY C LUKASKI

Start Date: 04/03/2004 **Term Date:** 01/25/2009

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Improve health and enhance quality of life by determining, for healthy and at-risk populations (e.g., school-aged children, rural elderly, Native Americans), mineral intakes that promote optimal physiological and psychological development, function and health; develop new functional bases for establishing mineral element requirements; identify mechanisms of action; and determine the influence of sex, age, genetic, lifestyle and environmental factors on mineral element requirements. Develop and implement health promoting interventions for prevention of obesity and co-morbidities in American Indian population in the upper Midwest.

1b. Approach (from AD-416)

Dietary intakes and biochemical indices of mineral status will be related to physiologic (e.g., body composition, weight maintenance, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine roles of specific minerals in supporting optimal function and development. A Mobile Nutrition Research Laboratory, Community Studies Unit, and a residential Metabolic Research Unit will be used to conduct epidemiologic, supplementation, fortification, and controlled feeding studies, respectively with healthy and at-risk subjects (e.g., school-aged children, rural elderly, Native Americans). Use qualitative assessment methods (focus groups and in-depth interviews) and surveys to develop and implement social ecological, culturally-sensitive and scientifically sound interventions in American Indian communities. Randomized controlled trials will evaluate the effects of graded intakes of minerals, such as iron, zinc, copper, mangesium and boron, and mediating factors (e.g., genotype, controlled stressors). Animal studies will be used to determine the mechanisms of action of functional outcomes. Studies will involve university, industry and government collaboration.

2. Milestones for FY2009

1. Conduct a pilot study of the physical activity components of the ARS Obesity Prevention Initiative Efficacy Study and analyze resultant data.
   Milestone Not Met
   Redirection (by Office of National Programs

2. Analyze and report data from studies, by using focus groups, surveys and other qualitative methods, the barriers and facilitators affecting Dietary Guideline adherence in preschool children, youth, college students, adult, and elderly, with a focus on American Indian communities.
   Milestone Substantially Met

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2009

NP / Component Coding

107	1	B	2009
107	1	C	2009
107	2	A	2009

4. Accomplishments

5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

01 KENYA

Collaboration with investigators at the U.S. Army Research Institute of Environmental Medicine (USARIEM) and the U.S. Army Medical Research Unit -Kenya and the Kenya Medical Research Institute/Walter Reed Project in a double-blind supplementation trial to test the hypothesis that zinc supplementation reduces the incidence and symptoms of diarrhea, and decreases the presence of bacterial vectors associated with diarrhea was completed (Cooperative Agreement 5450-51530-009-10R).

Scientific Publications: **Log 115**

1. Lukaski, H.C. 2008. Mineral Losses During Extreme Environmental Conditions. Cell Biology and Toxicology. 24:466-470. **000023290**

2. Lukaski, H.C. 2009. Evaluation of Body Composition: Why and How?. Mediterranean Journal of Nutrition and Metabolism. 2:1-10. **000023648**

3. Lukaski, H.C., Scrimgeour, A.G. 2009. Trace Elements Excluding Iron - Chromium and Zinc. In: Driskell, J.A. Nutrition and Exercise concerns of Middle Age. Baton Rouge, FL:CRC Press. p. 233-250. **000022255**

4. Nielsen, F.H. 2009. Major Minerals - Calcium, Magnesium, Phosphorus. In: Driskell, J.A. Nutrition and Exercise concerns of Middle Age. Baton Rouge, FL:CRC Press. p. 193-218. **000022312**

5. Scrimgeour, A.G., Lukaski, H.C. 2008. Zinc and Diarrheal Disease - Control Status and Future Perspectives. Current Opinion in Clinical Nutrition and Metabolic Care.1:711-717 **000023304**

6. Shafer, K.J., Siders, W.A., Johnson, L.K., Lukaski, H.C. 2009. Validity of segmental multiple-frequency bioelectrical impedance analysis to estimate body composition of adults across a range of body mass indexes. Nutrition. 25(1):25-32. **000021261**

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Prin Invs:** JANET ROSS HUNT

Start Date: 01/15/2004 **Term Date:** 01/14/2009

National Programs: 107 N Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered From: 10/2008 **To:** 9 /2009 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

The general objective is to determine how current and proposed changes to the American diet that may adversely affect intake and/or how bioavailability of the essential mineral nutrients can be modified to enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Specific objectives are:

Objective 1: Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. This objective will address the production of foods with enhanced bioactive Se compounds, and assess their ability to enhance health, especially by controlling oxidative stress and reducing cancer risk. The impact of organic farming methods will also be assessed (Finley). It will also address the practical impact of dietary changes that emphasize plant-based diets on meeting nutritional needs for Fe and Zn (Hunt).

Objective 2: Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. This objective will evaluate the bioavailability of Fe fortificants such as elemental Fe and micronized, encapsulated Fe compounds in human studies (Hunt).

Objective 3: Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components. Cell and whole animal models will be employed to elucidate how the modifications of mineral content of foods can influence the biochemical regulation of specific transporters, cellular trafficking, and interactions of minerals such as Zn, Fe, Cu, Cd, Se, and Mn. (Reeves).

Problem to be addressed with increased funds: Elucidate the roles and diets in support of optimal health and prevention of obesity and related illnesses, cardiovascular disease, osteoporosis and cancer.

Problem to be addressed with increased funds (FY05): Under Performance Measure 4.1.1 of the ARS Strategic Plan and the NP107 Action Plan, this project will develop an enhancement to the food supply by increasing the nutritional value of beef.

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2009

Objective modification FY05: Increase the amount of omega-3 fatty acids in beef to a nutritionally significant level by feeding flax. Demonstrate that the increase in omega-3 fatty acids in the meat are sufficient to have a physiological effect. Study feasibility of increasing selenium in beef to levels that will have an impact on human health when the meat is consumed at recommended levels. This may include studies of organic form of selenium in beef, stability with varying cooking methods, sensory issues, bioavailability and health effects in both steers and consumers.

1b. Approach (from AD-416)

Methodology will include tests of agricultural conditions affecting the amounts and forms of minerals incorporated into in foods; in vitro, cellular, and animal models of mineral transport and absorption; and human experiments with controlled diets to assess mineral absorption, retention, and biological function and to model nutritional requirements.

Specific objectives to be accomplished with increased funding: To study the roles of foods, particularly those produced in the Northern Plains, in the support of health. This work is to be multi-disciplinary, including collaborations such as with the University of North Dakota School of Medicine and Health Sciences and North Dakota State University.

2. Milestones for FY2009

1. Complete animal study of Se bioavailability from high-Se soy protein.
   Milestone Fully Met

2. Report high-Se soy protein bioavailability study.
   Milestone Substantially Met

3. Report study of phytate X Ca & Zn bioavailability.
   Milestone Fully Met

4. Complete final report of Fe excretion.
   Milestone Fully Met

5. Report study on validation of Caco-2 cell results with human absorption results in iron bioavailability from agricultural products.
   Milestone Fully Met

6. Report study of nonheme iron bioavailability from menus meeting the new Dietary Reference Intakes and Dietary Guidelines for Americans.
   Milestone Not Met
   Critical vacany (quantitative or qualitative deficiency in personnel

3. Progress Report

We conducted many studies to assess mineral utilization and bioavailability with changing diets and agricultural practices. A major accomplishment from the final year of this project is listed below. Other most significant accomplishments over the life of this project included - 1) Developed a mathematical algorithm to estimate dietary zinc absorption from food composition data, a useful tool to evaluate the diets of populations at risk of zinc deficiency; 2) Impaired iron absorption in copper deficient animals was associated with a reduction in copper containing hephaestin protein, copper deficiency impaired red blood cell formation, and excess dietary iron did not cure the anemia of copper deficiency. An understanding of the role of copper in iron metabolism and blood cell formation help set dietary copper recommendations; 3) Excess cadmium

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retention in animals marginally deficient in zinc, iron and calcium is independent of metallothionein protein, suggesting that metallothionein was not required to cadmium retention when it was fed to animals marginally deficient in zinc, iron and calcium; 4) Delineated mechanisms of antioxidant activity of sulforaphane and selenium from plant foods. An improved understanding of how diets influence oxidative stress helps determine dietary recommendations that can reduce the risk of chronic diseases; 5) Dry bean consumption improved blood cholesterol. These results are useful for setting dietary guidelines; 6) Serum pro-hepcidin, a peptide hormone, was not correlated with iron absorption in women, suggesting limitations of the commonly used assay of pro-hepcidin and the need of a more specific assay; 7) Selenium from high-selenium wheat was highly bioavailable, but it varied in different mill fractions of wheat; 8) Phytic acid inhibition of zinc absorption was not affected by dietary calcium fortification, suggesting that calcium in the range normally consumed did not interfere with zinc absorption, even at highly fortified levels; 9) Hemoglobin as the sole source of dietary iron did not support adequate iron status in rats, indicating that rat was not a good model for humans to study the utilization of iron from hemoglobin; 10) High bioavailability diets enabled humans to adaptively increase zinc absorption in response to low zinc intakes. These results are useful for setting dietary zinc recommendations and for improving human diets to reduce zinc deficiency; 11) Dietary guidelines emphasizing whole grains and other sources of phytic acid reduced iron absorption. It suggests a greater risk of dietary iron deficiency with the food recommendations and emphasizes the need to further modify and balance nutrients and food guideline to meet multiple nutritional goals. This research project will not be continued because of the Center's re-direction to obesity-related research. However, some results from this research project (e.g. high-selenium crops produced in Northern Plains) will be useful for a new research project, "Dietary Modulation of Obesity-Related Cancer by Selenium".

NP / Component Coding

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4. Accomplishments

01 Selenium from high-Se soybeans is highly bioavailable. Soy is a plant source of dietary protein and selenium is an essential mineral nutrient to humans. Northern Plains are rich in selenium in its soil, and soybeans produced in Northern Plains are high in this nutrient. We assessed bioavailability of selenium from the protein isolate and tofu (bean curd) prepared from a high-protein soybean cultivar developed for Nebraska producers and from a commercial line of soybeans produced in South Dakota. Selenium from these products was highly bioavailable to selenium-deficient animals compared with selenomethionine, a food form of selenium. These results will lead to further studies investigating the nutritional values and health benefits of high-selenium soybeans, and these results will also increase the marketability of soybeans produced in Northern Plains, particularly to selenium-deficient countries.

107 1 C 2009

5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

Scientific Publications: **Log 115**

1. Combs, G.F., Nielsen, F.H. 2009. Health Significance of Calcium and Magnesium: Examples from Human Studies. In: Cotruvo J., Bartram J., editors. Calcium and **000020567**

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2009

Magnesium in Drinking-water: Public health significance. World Health Organization Press. p. 85-95.

2. Hunt, J.R., Beiseigel, J.M. 2009. Dietary Calcium Does Not Exacerbate Phytate Inhibition of Zinc Absorption By Women From Conventional Diets. American Journal of Clinical Nutrition. 89(3): 839-843. **000023403**

3. Hunt, J.R., Johnson, L.K., Roughead, Z.K. 2009. Dietary Protein and Calcium Interact to Influence Calcium Retention: A Controlled Feeding Study. American Journal of Clinical Nutrition. 89:1357-1365. **000023443**

4. Hunt, J.R., Zito, C.A., Johnson, L.K. 2009. Body Iron Excretion by Healthy Men and Women. American Journal of Clinical Nutrition. 89(6):1792-1798. **000023561**

5. Murphy, K.M., Reeves, P.G., Jones, S.S. 2008. Relationship between yield and mineral nutrient concentrations is historical and modern spring wheat cultivars. Euphytica. 163:381-390. **000020439**

Approved: MCGUIRE MICHAEL R **Date:** 09/08/2009

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Prin Invs:** JANET ROSS HUNT

Start Date: 09/29/2003 **Term Date:** 09/28/2008

National Programs: 107 N Human Nutrition

Title: HUMAN STUDIES RESEARCH

Period Covered **From:** 10/2008 **To:** 9 /2009 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0324

Organization Name: UNIVERSITY OF NORTH DAKOTA

Progress and Outcomes:

1a. Objectives (from AD-416)

To investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function.

1b. Approach (from AD-416)

1. Provide expert guidance and technical support for human studies to elucidate functions of and quantitative needs for nutrients in maintaining health of adults through reduction of risk factors for cardiovascular disease, diabetes, cancer, osteoporosis and other degenerative diseases.
2. Plan and conduct human studies (including residential, non-residential and field-based investigations) using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses.
3. Design diets to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components.
4. Recruit, interview and screen volunteers for eligibility in human studies.
5. Determine clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function, respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status.
6. Publish scientific results.

3. Progress Report

This report documents research conducted under a Specific Cooperative Agreement between ARS and the UNIV OF NORTH DAKOTA. Additional details for the research can be found in the report for the parent project 5450-51000-035-00D, MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

The project involved six (6) new studies, one (1) survey and data analysis of 13 previously completed studies.

Found dietary selenomethionine increases biomarkers of selenium status. We completed a 1-yr trial to determine the relationship of selenium status and level of supplementation with a food form of selenium. Plasma selenium level responded linearly to increases in dietary selenomethionine intake adjusted for metabolic body size, which

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2009

we modeled. IMPACT: This algorithm will enable calculating the amount of dietary selenium needed to support selenium status for minimal cancer risk. This approach is needed to estimate the value of food sources of selenium in cancer prevention.

Found a high meat-protein diet with high potential acid load does not impair calcium retention in women. IMPACT: This study confirms previous work at the Center; it shows that diets high in meat protein do not impair the use of dietary calcium in healthy adults and refutes previous results with purified proteins in rats.

Found high bioavailability diets allow humans to adaptively increase their zinc absorption in response to low zinc intakes. Results gave a model to predict zinc absorption from dietary zinc and phytic acid. IMPACT: These results will assist in setting dietary zinc recommendations, which are based in part on predicted zinc absorption.

Found in a state-wide survey of 246 charitable food pantries and their users that the system served 8% of the ND population. User demand increases particularly among older and working-poor and clients in rural areas. IMPACT: The food pantry system may be an appropriate modality for health-based interventions.

Found regular physical activity induces beneficial changes in skeletal muscle that are limited by low copper intake. IMPACT: Individuals regularly engaging in exercise should ensure adequate copper intake.

Conducted pilot program of healthy food choices and exercise for 311 children enrolled in summer programs. This pilot showed the potential for nesting intervention within such a program.

Screened 415 candidates, enrolling 92 in a study to determine the effects of magnesium supplementation on magnesium status and sleep pattern.

ADODR monitoring included on-site visits and technical guidance, conference calls and meetings, e-mail and telephone communications.

Referred this FY-2009 ARS-421 project report to Marcie on 08/07/2009 for completion related to final administrative actions.

NP / Component Coding

Approved: MCGUIRE MICHAEL R **Date:** 10/08/2009

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2009

ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD **Prin Invs:** ERIC O UTHUS

Start Date: 07/21/2004 **Term Date:** 01/25/2009

National Programs: 107 N Human Nutrition

Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

Period Covered From: 10 / 2008 **To:** 9 / 2009 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Determine the molecular and cellular mechanism(s) of action of selenium (Se) in anti-carcinogenesis. Specific objectives include 1) Determine the role of Se in cell cycle progression and apoptosis in models of colon cancer; 2) Determine the role of selenoproteins in cancer prevention and the role of dietary components in the regulation of selenoprotein activity; 3) Determine the mechanism(s) by which Se alters DNA methylation and 4) Determine the relationship of oral selenium intake with selenium status and indicators of cancer risk.

1b. Approach (from AD-416)

A variety of cell culture and animal model approaches will be used. In general, cell culture experiments will be run using cell lines specific for colon. Various forms and concentrations of selenium will be added to serum-free media. Cell growth, indices of selenium status, and indices of cell cycle progression and apoptosis will be measured. These studies will be used to determine the effects of nutritional levels of selenium in supporting cellular survival signaling in human cultured colon cells, and the role of the putative anti-tumorigenic selenium-metabolite, methylselenol, in cell cycle progression and apoptosis in human cultured colon cells. Other cell culture models (colon and/or liver cell lines) will be used in siRNA knockdown studies. These experiments will determine the effect of selenium in cells in which specific genes have been knocked down by siRNA. Other studies will use knock downs of various selenoproteins to determine their role in anticarcinogenicity of selenium. Animal studies will use rats and mice to determine the effects of form and concentration of dietary selenium on 1) selenoprotein expression and activity as related to carcinogenesis, 2) carcinogen-induced aberrant crypt formation (preneoplastic colon cells) and, 3) indices of oxidative stress and one-carbon metabolism including DNA methylation of genomic and gene specific DNA.

2. Milestones for FY2009

1. Finish data collection and bioinformatics work on experiments designed to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium.
Milestone Fully Met

2. Complete aberrant crypt study and related methionine sulfoxide studies.
Milestone Substantially Met

3. Complete analytical work for selenium intervention trial.

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Milestone Fully Met

4. Use HPLC-ICP-MS method to assess selenium status in samples from selenium intervention trial.
Milestone Substantially Met

3. **Progress Report**

A number of studies were designed and undertaken to determine the mechanism(s) of chemoprevention by the nutrient selenium - major accomplishments from the final year of this project are listed below. Over the life of this project other major accomplishments included: 1) Development of animal models that were used to show that dietary obesity enhances secondary tumor development and tumor growth. Development of these animals models was crucial and will be used in studies on dietary intervention and prevention of obesity-related secondary cancer (future research project). 2) Methylselenol has been hypothesized to be a critical selenium metabolite for anticancer activity. We found that methylselenol increased the protein levels of two compounds that inhibit metastasis and inhibited the migration and invasion rate of fibrosarcoma cells in culture, thus decreasing the carcinogenic potential of activity of these tumor cells. 3) Our studies furthered the understanding of functional role of methylselenol as a cancer-chemopreventive compound as related to a high-fat diet. It is known that a typical Western diet is high in fat intake and that this leads to increased levels of fecal bile acids, and these bile acids, primarily deoxycholic acid in humans, are believed to be tumor promoters of colon cancer. We showed that methylselenol inhibits the growth of deoxycholic acid-resistant colon cells. 4) Our work showed that dietary selenomethionine increases biomarkers of selenium status in a predictable way and an algorithm was developed to facilitate calculating the amount of dietary selenium required to support selenium status associated with minimal cancer risk. This approach was required to understand the value of food sources of selenium in cancer prevention. 5) Other work with human volunteers showed that the glutathione peroxidase I genotype is a determinant of plasma selenium concentration. This finding is without precedent and challenges the assumption that plasma selenium necessarily predicts dietary selenium intake and, thus, has direct relevance to the design of dietary surveys including NHANES. 5) We found that butyrate treatment inhibits the migration and invasion potential of tumor cells - supporting the health claim of a high-fiber diet and providing a mechanism to link fiber and decreased cancer risk. 6) Our work with food forms of selenium resulted in a plausible mechanism to explain how broccoli consumption may inhibit cancer. 7) Some of our basic research in this project showed that dietary selenium impacts methionine metabolism and that dietary methionine can also affect selenium metabolism. This work is important to understand how different dietary factors can affect the anticarcinogenic nature of selenium. 8) Finally, our work with the long-lived Ames dwarf mouse gave insight to what mechanisms in animals are important in aging and in decreasing the risk of cancer. Results from this project are being used to provide the basis for a new project, "Dietary Modulation of Obesity-Related Cancer by Selenium".

NP / Component Coding

107	2	A	2009
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4. **Accomplishments**

01 Diet-induced obesity enhances secondary tumor formation: Obesity is a risk factor of cancer, not only of primary cancer but also of secondary cancer which results in poor prognosis in cancer patients and affects survival rate and quality life of cancer survivors. ARS scientists in Grand Forks, ND have successfully demonstrated that diet

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induced obesity enhanced secondary tumor development and growth in animals using different models that assessed different steps of this malignant process. Results frc these studies will be very useful to further studies on the roles of dietary intervention and/or physical activity on obesity-related secondary cancer development and growth.

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02 The selenium metabolite, methylselenol, reduces invasive potential of tumor cells: Ir humans and animals, cell proliferation and cell death must be regulated to maintain tissue homeostasis. It is well documented that the abnormal control of cell cycle progression and cellular death is directly related to cancer. ARS scientists in Grand Forks, ND demonstrated that a very small amount of methylselenol inhibits the migration and invasive potential of tumor cells by inhibiting certain cancer signalir pathways. This provides a mechanism of selenium's anticarcinogenic actions. These dat will help in determining the molecular basis of obesity-related secondary cancer and aid in dietary intervention and prevention of cancer.

107 2 A 2009

03 ARS scientists in Grand Forks, ND developed an algorithm based on empirical observations to predict the quantitative effect of dietary selenium intake on seleniu status in healthy Americans. A year-long, randomized, multi-dose, clinical trial was conducted to elucidate the relationship of dietary selenium intake on the steady stat level of plasma selenium. The work showed that dietary selenomethionine increases biomarkers of selenium status in a predictable way; an algorithm was developed describing that relationship. This algorithm can be used to determine the amount of dietary selenium required for individuals of known selenium status to achieve status associated with cancer risk reduction. This will facilitate the development and use c food sources of selenium for cancer prevention. The scientists also discovered genotypes associated with cancer risk (the antioxidant enzyme glutathione peroxidase and the major selenium transporter selenoprotein P) affect plasma selenium level and urinary selenium excretion. These findings are without precedent and associate aberrant selenium metabolism and cancer risk.

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5. Significant Activities that Support Special Target Populations

7. International Cooperation / Collaboration

01 COSTA RICA

Costa Rica - collaborated with researchers at the University of Costa Rica in the first-ever survey of selenium status in healthy children and mothers in that country. No agreement.

02 GERMANY

Germany - collaborated with researchers at the German Endocrinology Institute, Berlin, to determine selenoprotein P levels in the plasma of healthy Americans. No agreement.

Scientific Publications: **Log 115**

1. Carlson, B.A., Schweizer, U., Perella, C., Schrimali, R.K., Feigenbaum, L., Shen, L., Speransky, S., Floss, T., Jeong, S., Watts, J.J., Hoffman, V., Combs, G.F., Gladyshev, V.N., Hatfield, D.L. 2009. The Selenocysteine tRNA STAF-Binding Region is Essential for Adequate Selenocysteine tRNA Status, Selenoprotein Expression and Early Age Survival of Mice. Biochemical Journal. 418:61-71. 000023312

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2009

2. Combs, G.F., Midthune, D.N., Patterson, K.K., Canfield, W.K., Hill, A.D., Levander, O.A., Taylor, P.R., Moler, J.E., Patterson, B.H. 2009. Effects of Selenomethionine Supplementation on Selenium Status and Thyroid Hormone Concentrations in Healthy Adults. American Journal of Clinical Nutrition. 89(6):1808-1814. 000020629

3. Hu, H., Li, G., Wang, L., Watts, J.J., Combs, G.F., Lu, J. 2008. Methylseleninic Acid Enhances Taxane Drug Efficacy against Human Prostate Cancer and Down-Regulates ntiapoptotic roteins Bcl-XL and Survivin. Clinical Cancer Research. 14(4):1150-1158. 000021426

4. Li, G., Lee, H., Wang, Z., Hu, H., Liao, D.J., Watts, J.J., Combs, G.F., Lu, J. 2008. Superior In Vivo Inhibitory Efficacy of Methylseleninic Acid Against Human Prostate Cancer over Selenomethionine or Selenite. Carcinogenesis. 29(5):1005-1012. 000021427

5. Uthus, E.O., Ross, S. 2008. Dietary selenium (Se) and copper (Cu) interact to affect homocysteine metabolism in rats. Biological Trace Element Research. Available: http://www.springerlink.com/content/ 000023168

6. Yan, L., Spitznagel, E.L. 2009. Soy Consumption and Prostate Cancer Risk in Men: A Revisit of Meta-Analysis. American Journal of Clinical Nutrition.89:1155-1163. 000023288

7. Zeng, H. 2009. Selenium as an Essential Micronutrient: Roles in Cell Cycle and Apoptosis. Molecules. 14(3):1263-1278. 000023605

8. Zeng, H., Botnen, J.H. 2008. Selenium is critical for cancer-signaling gene expression but not cell proliferation in human colon Caco-2 cells. Biofactors. 31(2007):155-164 000021788

9. Zeng, H., Botnen, J.H., Johnson, L.K. 2008. A selenium-deficient Caco-2 cell model for assessing differential incorporation of chemical or food selenium into glutathione peroxidase. Biological Trace Element Research. 123:98-108. 000021066

10. Zeng, H., Uthus, E.O., Ross, S., Davis, C.D. 2009. High Dietary Intake of Sodium Selenite Does Not Affect Gene Mutation Frequency in Rat Colon and Liver. Biological Trace Element Research. DOI 10.0017/s/12011-009-8348-3. Available: http://www.springerlink.com/humana+press/biochemistry.journal/12011. 000023185

Approved: MCGUIRE MICHAEL R **Date:** 09/02/2009